EFFECT OF ENVIRONMENTAL SALINITY ON THE APICAL SURFACE OF CHLORIDE CELLS OF THE EURYHALINE TELEOST, *MUGIL PLATANUS* (PISCES, MUGILIDAE)

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ABSTRACT

Juvenile mullets *Mugil platanus* (Günther, 1880) were transferred from saltwater to waters with decreasing salinities in order to observe changes in the gill epithelium morphology, mainly at the apical surface of chloride cells (CCs) of the afferent filament side. The openings of juveniles CCs adapted to sea water are narrow and deep among the borders of adjacent pavement cells with ∅ = 0.5 - 1µm. Changes in ultrastructure were observed within 15 min after the transfer to freshwater. At first, the surface of openings became larger, usually with ∅ = 0.5 - 1µm, and exhibited microvilli. Since openings with different sizes were present after 6 h in freshwater, the response of the operings of CCs in *M. platanus* adapted to freshwater was not an all-or-none phenomenon.

KEYWORDS. Gill arch, chloride cells, *Mugil platanus*.

INTRODUCTION

Chloride cells (CCs) appear to be responsible for the osmotic regulation of fish adapted to sea water and freshwater due to their columnar position in the epithelia, the high number of mitochondria in the cytoplasm, the basolateral membranes in close association with the mitochondria and the presence of Na-K-ATPase in intimate association with the tubular system membrane (Pisam, 1981; Karnaky **et al.**, 1984).

Another strong evidence for the participation of CCs in the osmoregulation processes is the ultrastructural modification associated with the Na-K-ATPase enzyme, that occurs when the fish adapt to environmental changes. During the adaptation of euryhaline fish to sea water, the following has been observed: (1) an increase in enzymatic activity (Hossler **et al.**, 1979; Epstein **et al.**,1980), (2) an increase in size and number of CCs and mitochondria (Shirai & Utida, 1970; Pisam, 1981), (3) a greater development of

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the tubular system (SHIRAI & UTIDA, 1970) and (4) a development of accessory CCs forming "leaky junctions" (PISAM **et al.**, 1990). During adaptation to freshwater, the process is inverted and a decrease in enzyme activity occurs, as well as in the number and size of CCs and mitochondria, in the tubular system (SHYRAI & UTIDA, 1970; EVANS, 1993) and in general the intercellular complexe between the CCs and the accessory CCs disappears.

Several studies have been performed to evaluate the effect on CCs of euryhaline fish in distinct environments. However, the majority of these studies consisted on adapting freshwater fish to sea water and only few have attempted the opposite. This study, using scanning electron microscopy (SEM), aims to show apical changes of CCs the gill epithelia of young mullets *Mugil platanus* (Günter, 1880),when submitted to decreasing salinity gradients.

MATERIAL AND METHODS

Young *Mugil platanus* of 28 to 33 mm were captured during the summer of 1995, using a 7.0 m x 2.0 m beach seine net and 1 mm wire mesh in creeks situated on the beach at Pontal do Sul, Paraná, Brazil. They were transported and maintained in a controlled temperature chamber at 25° C, during 7 days in fiber tanks with 300 L of sea water with the same salinity of the collection area. During this period, fish were fed once a day with pellet rations such a Pirá Tropical Growth in a proportion equivalent to 20% of the tank biomass.

After the acclimation period, 20 individuals were transferred directly to buckets containing 16 L of water with salinities of 34‰ (control), 15‰, 5‰, 3‰, 1‰ and 0‰ (freshwater), including a replica for each salinity. Throughout the experiments the water was aerated and the fish were not fed. At the end of each sampling, dead fish were removed, the detritus were siphoned and the water level was readjusted. Individuals that showed signs of stress, such as a darkened dorsum, irregular swimming, or those which remained immobile on the bottom or moved only when stimulated mechanically, were not used for the chloride cell analysis.

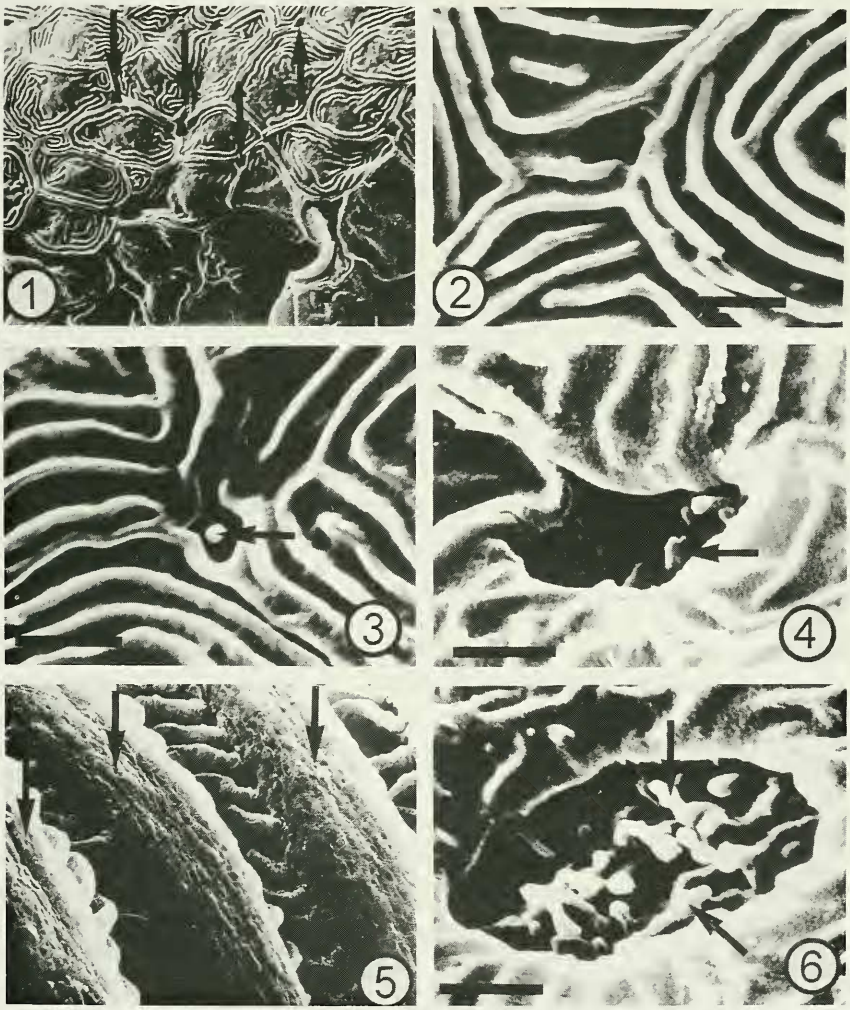
From each bucket two individuals were collected in a time frame of 1, 3, 6, 12, 24, 48 and 96 h after the beginning of the experiment. In the 0 ‰ salinity, observations were made at 15, 30, 45 min and 1, 3 and 6 h. The fish were decapitated and the gill arches were carefully removed from the opercular cavities. In the 34‰ and 15‰ salinities, the arches were rinsed with a Curtland solution. In the other salinities, the arches were rinsed with a saline solution (NaCl 0.9%). The second left gill arch was removed and fixed for 24 h at 4-5 °C with 3% glutaraldehyde buffered with 0.2 M sodium cacodilate (pH 7.2), and then rinsed. They were to 50% ethanol for 15 min and then preserved in 75% ethanol at a temperature of 4-5°C. In preparation for the SEM, then arches were successively transferred to 90% ethanol and two series of 100% ethanol and maintained for 15 min in each concentration, dehydrated with CO_2 and sputter coated on an aluminum support. The observations and electron micrographs were perfomed with Scanning Eletronic Microscopes Phillips (SEM 505) and JEOL (JSM 840 A). The electron microgaphs were classified according to salinity and time treatments and the largest and smallest axis of the CC openings were measured. In order to test the hypothesis that the openings do not vary according to time and salinity, the t-test and two-way analysis of variance were used. When significant differences were observed ($p \leq 0.05$), a test of minimum significant difference was used (SNEDECOR & COCHRAN, 1980).

The material is deposited in the reference collection of the Ichthyoplankton Laboratory , Centro de Estudos do Mar, Universidade Federal do Paraná, Pontal do Sul.

RESULTS

In all the salinities used, many openings were randomly distributed among the pavement cells, in the afferent side at the base of the secondary lamellae and in the interlamelar region of the gill filament epithelium (fig. 1).

The mean, standard deviation and range values of the large and small axis of the chloride cells are shown in table I. Alterations in the surface ultrastructure of the epithelial



Figs. 1-6. Gill filaments of *Mugil platanus* exposed to 34, 3, 1 and 0% salinities: 1, afferent region of filament showing CC openings in 34% salinity after 96h; 2, detail of CC opening in 34% salinity after 96 h; 3, detail of CC opening in 3% salinity after 1 h. Note the presence of microvilli; 4, detail of CC opening in 1% salinity after 12 h. Note the presence of mocrovilli; 5, afferent region of filament showing CC openings in 0% salinity after 30 min; 6, detail of CC opening with many microvilli in 0% salinity after 1 h. Bars 1µm.

Table I. Range, mean and standard deviation of the large and small axis of the chloride cell openings in gills of juvenile *Mugil platanus* in all salinities during 96h experiment.

Salinity	Large axis (µm)	Mean (µm)	Small axis (µm)	Mean (µm)
34‰	0,50 - 1,50	0,82 ± 0,24	0,50 - 1,00	0,70 ± 0,24
15‰	0,50 - 1,50	0,95 ± 0,27	0,50 - 1,50	0,79 ± 0,26
5‰	0,50 - 2,00	0,95 ± 0,28	0,50 - 1,00	0,77 ± 0,24
3‰	0,30 - 3,00	1,02 ± 0,41	0,30 - 2,50	0,73 ± 0,26
1‰	0,50 - 3,50	1,15 ± 0,52	0,30 - 2,50	0,87 ± 0,37
Freshwater	0,50 - 5,00	1,96 ± 0,89	0,50 - 4,00	1,35 ± 0,65

openings after 96 h in 34‰, 15‰ and 5‰ salinities were not significant (fig. 2).

In the 3‰ salinity, the openings showed some alteration in the size and microvilli appear after 1h of experiment (fig. 3). The fish in 1‰ salinity showed larger openings and the microvilli were more frequent (fig. 4). In freshwater, after 15 min of experiment, openings with increased dimensions and microvilli were clearly visible. (figs. 5,6).

A two-way analysis of variance in the larger axis showed significant differences between salinities, time, and an interaction between these two factors. A multiple comparison test of minimum significant differences (MSD) showed that this axis obtained greater mean lengths in 0‰ salinity, followed by 1‰, both being larger than in all other salinities. Non-significant differences were found between the mean values of 3‰, 5‰ and 15‰ salinities, and the mean values in the 5‰ and 15‰ salinities were not significantly different from the 34‰ salinity means. Regarding the mean length of the larger axis throughout the time frame, the MSD showed a significantly greater mean value after 6h of experimentation. Significant differences did not occur between the values of the other time frames (tab. II).

Table II. Results of two-way analysis of variance applied in the greater and lesser axis of the CC openings in *Mugil platanus*, comparing salinities and times. Results of significant differences by multiple comparison of minimum significant differences test (MSD). NS- non-significant difference; *P ≤ 0.05.

	LARGER AXIS F	SMALLER AXIS F
SALINITY (SAL)	3.65 *	1.95 NS
TIME (T)	3.28*	4.28*
INTERACTION	3.00*	2.45*

MSD LARGER AXIS	SAL 0‰	SAL 1‰	SAL 3‰	SAL 5‰	SAL 15‰	SAL 34‰
	T6	T1	T3	T12	T24	T48 T96

Concerning the smaller axis, a two-way analysis of variance pointed to significant

differences between time and to the interaction between the effects of salinity and time, with no significant differences between salinities. The use of the multiple comparison test of minimum significant difference pointed to a greater mean length after 6 h of experimentation. Moreover the averages at 1, 3 and 48 h are similar to those at 96 h, which are significantly greater than the means at 12 and 24h of the experiment (table II).

DISCUSSION

The morphological changes of CCs are perhaps the best documented responses for the transfer of a euryhaline fish from freshwater to sea water and vice versa. While Kessel & Beams (1962) and Philpott & Copeland (1963) did not observe evident changes in CCs when exposed to sea water, other authors noted an increase in their number and size (Shirai & Utida, 1970; Pisam, 1981; Pisam **et al.**, 1987).

According to Maina (1990) the adaptation of a fish to a new environment occurs during a brief period, being faster in freshwater than in sea water. In juveniles of *M. platanus*, there was a rapid response to the reduction of salinity mainly in freshwater with concentrations under 5%, where a noted increase of the openings in the CCs was observed after only 15 min of experimentation. Rapid alterations were also observed in *Mugil cephalus* Linnaeus, 1758 (Hossler, 1980), in which changes in the openings occured after 3 h in freshwater. Slower responses were observed in *Lebistes reticulatus* Peters, 1859 (Straus, 1963) and *Anguilla japonica* Temmick & Schlegel, 1912 when transferred to freshwater (Shirai & Utida, 1970). Such differences could be correlated with the osmoregulatory ability of the fish (Hwang & Hirano, 1985).

In freshwater-adapted teleosts, the CCs showed openings with large diameters containing many microvilli (Philpott & Copeland, 1963; Hossler, 1980). These alterations of the CC openings were observed in juvenile *M. platanus* and were similar to changes previously reported fo *M. cephalus* by Hossler **et al.** (1979) and Hossler (1980).

Hossler (1980) observed that in *M. cephalus*, maintained in 10‰ salinity, the openings of the CCs measured from 1 to 5 µm and showed numerous microvilli on their surface. After 24 h in freshwater, the openings had a mean diameter of 3-6µm. Considering the differences among species and fish size, the changes observed in this study were different from those observed by Hossler (1980) in *M. cephalus*. In *M. platanus* the openings measured 0.50 x 1.50µm in sea water and 0.50 x 5µm in freshwater.

Maina (1990) observed that the morphological modifications of the CCs in *Oreochromis alcalicus grahami* Boulenger, 1910 are not an all-or-none phenomenon, and that the degree of responses is dependent on the salinity gradient of the environment, on the species, and on the stage of development. The different levels of morphological modifications of CCs from the gill of *M. platanus* submitted to the same treatment appear to agree with the statement that the CCs' responses are not uniform.

When the fish are transferred to freshwater, the morphological responses may be the consequence of physiological response. Epstein **et al.** (1980) transferred *Anguilla* sp. adapted to sea water, to freshwater for only 2 h, and observed a sudden drop in flux rates of Na^+ and Cl^-, without hte concomitant alteration in Na^-K^-ATPase. This suggests that this flux change could be the result of the effect of hormones (prolactin) on the "leaky junctions" of the CCs-CCs or on the permeability of the apical membrane of the CCs, providing an immediate protection against demineralization. This immediate protection

could explain the absence of deaths among the juveniles of *M. platanus* transferred to 15‰ and 5‰ salinities. Although morphological variations were present, they did not show significant differences from the control group. According to HWANG & HIRANO (1985) the intermediary salinities provide only part of the CCs' responses. Such hormonal processes, as well as the substantial and rapid alterations in CC openings, do not appear to be sufficient for the survival of the juvenile *M. platanus* transferred directly to 3‰, 1‰ and 0‰ salinities.

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REFERENCES

EPSTEIN, F.H.; SILVA, P. & KORMANIK, G. 1980. Roles of Na-K-ATPase in chloride cell function. **Am. J. Physiol.**, Bethesda, **238**: 246-250.

EVANS, D.H. 1993. Osmotic and ionic regulation **In**: EVANS, D.H. ed. **The physiology of fishes**. Florida, CRC p. 315-342.

HOSSLER, F.E. 1980. Gill arch of the mullet, *Mugil cephalus*. III. Rate response to salinity change. **Am. J. Physiol.**, Bethesda, **238**:160-164.

HOSSLER, F.E.; RUBY, J.R. & MCWIWAIN, T.D. 1979. The gill arch of the mullet, *Mugil cephalus*. II. Modification in surface ultrastructure and Na-K-ATPase content during adaptation to various salinities **J. exp. Zool.**, New York, **208**:399-406.

HWANG, P.P. & HIRANO, R. 1985. Effects of environmental salinity on intercellular organization and junctional structures of chloride cells in early stages of teleost development. **J. exp. Zool.**, New York, **236**:115-126.

KARNAKY, K.J. JR.; DEGNAN, K.J. **et al.** 1984. Identification and quantification of mitochondria-rich cells in transporting epithelia. **Am. J. Physiol.**, Bethesda, **246**:770-775.

KESSEL, R.G. & BEAMS, H.W. 1962. Electron microscope studies on the gill filaments of *Fundulos heteroclitus* from sea water and fresh water with special reference to the ultrastructural organization of the "chloride cell". **J. Ultrastruct. Res.**, Orlando, **6**:77-87.

MAINA, J.N. 1990. A study of the morphology of the gills of an alkalinity and hyperosmotic adapted teleost *Oreochromis alcalicus grahami* (Boulenger) with particular emphasis on ultrastructure of the chloride cells and their modifications with water dilution. A SEM and TEM study. **Anat. Embryol.**, New York, **181**:83-98.

PHILPOTT, C.W. & COPELAND, D.E. 1963. Fine structure of the chloride cells from three species of *Fundulus*. **J.Cell Biol.**, New York, **18**:389-404.

PISAM, M. 1981. Membranous system in the chloride cells of teleostean fish gill: their modifications in response to the salinity of environment. **Anat. Rec.**, New York, **200**:401-414.

PISAM, M. CAROFF, A. & RAMBOURG, A. 1987. Two types of chloride cells in the gill of freshwater-adapted euryhaline fish: *Lebistes reticulatus*: their modifications during adaptation to saltwater. **Am. J. Anatom.**, New York, **179**:40-50.

PISAM, M.; BOUEF,G.; **et al.** 1990. Ultrastructural features of mitochondria-rich cell in stenohaline freshwater and sea water fishes. **Am. J. Anatom.**, New York, **187**:21-31.

SHIRAI, N. & UTIDA, S. 1970. Development and degeneration of the chloride cell during sea water and fresh water adaptation of the japanese eel *Anguilla japonica*. **Z. Zellforsch. mikrosk. Anat.**, Berlin, **103**: 247-264.

SNEDECOR, G.M. & COCHRAN. W.G. 1980. **Statistical methods**. 7 ed. Iowa, Iowa State Univ. 507p.

STRAUS, L.P. 1963. A study of the fine structure of the so-called chloride cell in the gill of the guppy *Lebistes reticulatus*. **Physiol. Zoöl.**, Chicago, **36**(3):183-198.

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